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Analysis of response to water deficit in three Indian varieties of chickpea (*Cicer arietinum* L.) for drought tolerance

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Abstract

Drought is one of the major abiotic stresses in agriculture for losses in crop productivity worldwide. Three chickpea (*Cicer arietinum* L.) varieties namely P362, P1103 and SBD377 were assessed for response to drought tolerance during vegetative stage, in stress and non-stress environments, under contained conditions. Several physiological parameters including gas exchange, photosynthesis rate, fluorescence, stomatal conductance and water loss per day were monitored simultaneously. P362 variety showed maximum photosynthesis rate in irrigated as well as in drought conditions. This variety also maintained its relative water content (RWC) and water potential (WP) during imposition of similar duration of drought. Due to the maximum elasticity of leaf cells, it maintained its cell turgidity upto 68% RWC to protect itself from water stress, compared to variety P1103 and SBD377. The effective solute concentration and osmotic potential in the irrigated controls at full turgor was lowest in P362 variety, compared to the other two varieties. Osmotic adjustment (OA) was assessed as a capacity factor which is rate of change in turgor pressure with RWC. P362 variety showed a maximum OA value of 0.27 while the values for SBD377 and P1103 were 0.22 and 0.21, respectively. During water stress, the chlorophyll content was minimally reduced in P362 variety, therefore effective quantum yield of photosystem II (F_v/F_m) and photosynthesis rate was maximally maintained. The higher photosynthesis rate under irrigated conditions and maintenance of higher RWC under drought conditions makes P362 variety a promising option for optimum yield under prolonged terminal drought or under rain-fed conditions.

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Introduction

The land plants have been coping with water stress, ever since they left the seas and colonized the dry land (Thomas 1997). As time passed by, progressive anthropogenic activities of the modern era has made the weather more unpredictable and crop plants dependent on rainwater are still facing the vagaries of the ever changing weather conditions. Because, land plants experience constant fluctuations in the availability of water, they have evolved adaptive features to search for and absorb water through their root systems, to prevent excessive transpirational water loss and to adjust their physiology and biochemistry for survival and sustainable growth and (Zhang *et al.,* 1996; Zhu *et al.,* 1997).

Chickpea (*Cicer arietinum* L.) is an ancient legume crop believed to have originated in South Eastern Turkey and adjoining parts of Syria (Singh 1997). It is the second most important pulse crop of the world and covers 15% of the cultivated area thus, contributing to 14% (7.9 million tonnes) of the world's total pulses productivity of 58 million tonnes. India is the largest producer of chickpea in the world but the yield has been stagnating for last two decades primarily due to abiotic and biotic stresses and relatively slow progress in its genetic improvement (Dita *et al.,* 2006; FAO 2012).

Chickpea plays a significant role in the nutrition of both rural and the urban population in the developing world. Improving its adaptation to drought including terminal drought is critical for sustained grain yield under rain-fed cultivation. From an estimated 3.7 million tonnes annual loss in chickpea through water deficit in semi-arid regions, about 2.1 million tonnes could be recovered by crop improvement efforts (Bhatnagar-Mathur *et al.,* 2009). However, the multigenic and quantitative nature of drought tolerance makes it difficult to increase abiotic stress tolerance using conventional plant breeding methods and availability of genotypes tolerant to drought (Singh *et al.,* 2012). Unfortunately, cultivated chickpea has high morphological but narrow genetic diversity and understanding the genetic processes of this plant is hindered by the fact that its genome has not yet been annotated for adequate EST and SNP resources (Varshney *et al.,* 2013; Jain *et al.,* 2013). Although, chickpea is considered as drought-tolerant cool-season food legume but terminal drought still limits chickpea production and grain yield. Due to terminal drought, seed yield can be reduced by 58–95% compared to irrigated plants with reduction in pod production per plant and abortion are the chief factors affecting the overall grain yield (Behboudian *et al.,* 2001; Leport *et al.,* 2006).

In chickpea, a deep root system, osmotic adjustment, high leaf water potential, early flowering and maturity, high biomass, and apparent redistribution of stem and leaf dry matter during pod filling are associated with drought tolerance (Morgan *et al.,* 1991; Subbarao *et al.,* 1995; Leport *et al.,* 2006). The requirement of water during flowering, pod development and seed filling stages is crucial for the productivity of chickpea plant. The influence of drought on yield of chickpea has been documented, but extensive research on the physiological responses of water stress on chickpea is limited (Sheldrake and Saxena 1973; Turner and Begg 1981). Leaf water potential is a good indicator of plant water stress and correlates well with different plant functions and crop productivity in legumes (Sojka and Parsons 1983; Phogat *et al.,* 1984)

Three chickpea varieties P362, P1103 and SBD377 were grown for the assessment of drought stress response under water deficit and non-stress environments. Various physiological parameters like plant water loss per day, plant height, total photosynthesis area, relative water content, plant water potential, gas exchange, fluorescence and wet sensor reading of soil parameters were assessed. Based on these physiological parameters, the best responding variety to drought stress environment was determined during the course of the study, which can be incorporated in chickpea breeding programmes for the introgression of drought tolerance trait in other high yielding but drought sensitive varieties for cultivation in rain fed areas and genetic improvement of chickpea for drought tolerance.

Material and Methods

Plant material

Chickpea (*Cicer arietinum* L.) genotypes P362, P1103 and SBD377 seeds were obtained from Indian Agricultural Research Institute, New Delhi. After thorough washing, the seeds were soaked overnight in (RO) reverse osmosis purified water and were germinated in pots in a glass house maintained with a day/night cycle of 14 h/10 h at 28 °C/20 °C. When grown for 30 days, the potted plants of the varieties were separated into two groups of 10 each: one group was watered and used as control and second group was subjected to water stress. The youngest fully-expanded leaf (second or third from the apex) was used for various physiological measurements.

Drought experiments

The drought experiments were performed in a glass-house at CSIR-NBRI, Lucknow. Total of 60 pots were watered to pot capacity, weighed and kept for three days to measure the soil water holding capacity. After transplanting one month old chickpea plants of each variety, the pot weight was maintained at 1.5 kg. During drought experiments, water was withheld from half of the pots (water stressed, WS), while others were kept well watered (WW), everyday, to maintain the optimized weight. The pots for drought experiments were covered with rexine sheet to prevent soil-water loss by evaporation. No chemical fertilizer was added to the soil or sprayed on the leaves so as to mimic the natural growth conditions.

Total leaf area, plant height and chlorophyll content

The total leaf area (cm^2) and plant height (mm) of the three chickpea varieties was recorded weekly, throughout the drought experiments. A separate timeline graph of leaf area and plant height was plotted against the days of observation, for control plants and plants under test. Leaf chlorophyll content was also determined using 80% acetone and calculated as mg g^{-1} fresh weight. After centrifugation (20,000 × *g*, 20 min) the absorbance was read spectrophotometrically at 663 and 645 nm. Total chlorophyll as well as chlorophyll *a* and *b* concentrations were calculated according to Arnon, 1949.

Measurement of relative water content (RWC)

The amount of water in plant material can be expressed in a number of ways. All are based on the measurement of fresh weight (FW) at the time of sampling, dry weight (DW), usually oven dry weight at 80 °C for three days and turgid weight (TW). The RWC of the leaves of three chickpea varieties was tabulated weekly and calculated as percentage of RWC (% RWC).

$$\% \text{ RWC} = (\text{FW-DW/TW-DW}) \times 100$$

Water potential (Ψ) measurement and pressure volume (PV) curve derivatives

About 15–20 mg of fresh leaves was immersed in water for 15–30 min to obtain fully turgid leaves. Then leaves were blotted thoroughly using paper towels until they released almost no water, quickly weighed and placed in psychrometer chambers of Psypro Water Potential System (Wescor, USA). After equilibration for 4 h, chambers were connected to a Wescor HR-33T micro voltmeter and water potential was measured. Leaves were then allowed to lose about 5–20% of their water and allowed to equilibrate again. Measurements were repeated until the water potential (Ψ) fell to about -5 MPa. Leaf weight was taken after drying for 70 h at 50 °C in hot air oven. Psychrometer chambers were calibrated with a standard solution of 0.5 M NaCl at 25 °C.

A typical PV curve was drawn by plotting 1/Ψ against leaf RWC (Beckett 1997). The resulting curve was initially concave but beyond the region where turgor is lost (i.e. where turgor no longer contributes to Ψ) the curve became linear. From the PV isotherm, turgor potential was calculated as the difference between the extrapolated linear portion of the curve and the actual curve and turgor pressure (TP) was then plotted as a function of RWC. Osmotic potential (OP) at full turgor was calculated as the y-intercept of the linear portion of the PV curve. Regression line going through linear portion of the curve intercepts at x-axis and yields the symplastic and apoplastic fraction of water. Tissue elasticity was calculated from the relationship between WP and RWC (Stadelmann, 1984). The bulk elasticity modulus (ε) of tissue expresses the change in turgor of tissue cells (dP) for a unit change in the relative water content (dr) of the cells (ε = dP/dr). Osmotic adjustment was assessed as the capacity factor (rate of change in turgor pressure with RWC), by regression of RWC versus turgor pressure as described by Kumar and Singh, 1998. The reciprocal of slope (p) is, therefore, a measure of osmotic adjustment.

Gas exchange measurements

The gas exchange and chlorophyll fluorescence parameters were measured weekly in plants under irrigated and stressed conditions with an open infrared portable gas-exchange fluorescence system (GFS-3000; Heinz Walz GmbH, Germany) equipped with a clear top cuvette, standard measuring head 3010-S with leaf area adapter 3010-2 × 4 and PAM-fluorometer 3050-F” fiber optics probe, under ambient temperature, vapour pressure deficit (VPD) and photosynthetic photon flux density (PPFD) conditions. During gas exchange measurements, the flow rate of air through the cuvette was maintained approximately to ambient CO_2 (ranging between 375 and 385 μmol mol^{-1}). The fourth leaf from the top was selected for various studies. The attached leaflets were enclosed in an 8 cm^3 plexiglass chamber. The rates of photosynthesis and transpiration were observed after reaching steady-state condition about 20 min. The various chlorophyll fluorescence parameters including the effective quantum yield of PSII (Φ), apparent electron transport rates through PSII (ETR), photochemical quenching (qP), non-photochemical quenching (NPQ) and the maximum quantum yield of PSII (Fv/Fm) were calculated as described by Maxwell and Johnson, 2000.

Soil water parameters

WET-sensor (Delta-T Devices, Cambridge, UK) was used to measure soil volumetric water content (θ), bulk electrical conductivity (EC_b) and soil temperature. The dimensions of the housing were 46 mm x 55 mm x12 mm, and the electrodes had a length of 68 mm, each spaced 15 mm from each other. Since the water stress experiments were conducted in a contained glass house, the soil temperature was maintained between 20–23 ^{o}C and is therefore not described further. The permittivity (ε) of soil is calculated as $\varepsilon = \varepsilon' - j\varepsilon''$, where the real part of permittivity, ε', represents the energy stored and the imaginary component, ε'', represents the total energy absorption or loss.

Statistical analysis

Each experiment was performed thrice with five replicates. The mean values (± the standard error, SE) obtained in one experiment with five replicates are shown in the figures. Data were analyzed using Student’s t-test. All the graphs were prepared using Sigma Plot software (Sigma Plot, USA).

Results

Plant phenotype

Chickpea plants grown and incubated Plant incubated under control conditions have shown normal healthy growth with dark green turgid leaves and average number of 14–16 ± 2 pods per plant compared to water stressed plants for 30 days were bearing lesser number of leaves, pods and weak in appearance. During the water stress experiments the seed number was affected in all the three varieties because of fewer pods formed after the imposition of water stress. Interestingly, water stress did influence seed number but not the seed mass. Our aim was to screen out the best tolerant variety and the phenological analysis of chickpea plants of three varieties, under irrigated and water stress conditions that have clearly mirrored the extent of tolerance of variety P362 to water stress (Fig. 1).

Fig. 1 Plants of three chickpea varieties under control (C) and after 30 days of experimental drought (D) conditions. (*a*) P362 (*b*) P1103 and (*c*) SBD377.

Water loss per day

As mentioned in the methodology section, each pot was weighed daily in the morning and evening and the weight of the well watered (WW) pots were maintained to 1.5 kg. The average water loss per day versus days of drought is shown in Fig. 2A and B.

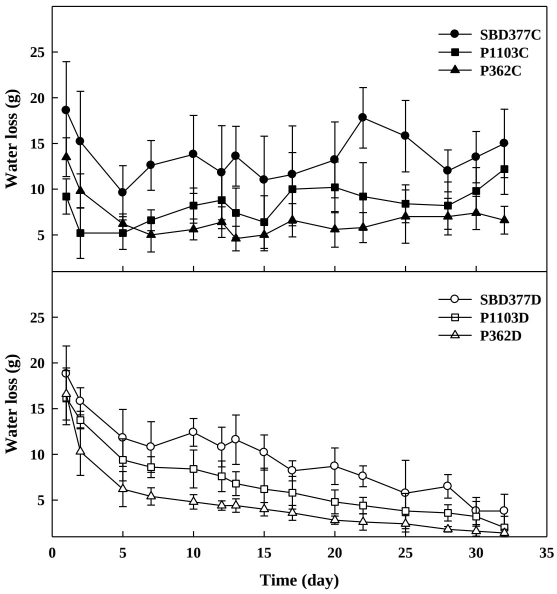

Fig. 2 The average water loss in 24 h during irrigated and drought conditions. (*a*) Average water loss per day of ten chickpea control plants of each variety. (*b*) Average water loss per day of ten plants of each variety under drought.

Terminal drought treatment was given for 30 days to the plants of three varieties P362, SBD377, P1103 and results have shown that P362 looses less water during water stress, due to controlled transpiration rate, than the other two varieties and can be represented as P362 < P1103 < SBD377. In irrigated as well as drought conditions SBD377 variety lost maximum water through transpiration followed by P1103 and P362. The water loss in irrigated controls was not significant, while in drought conditions the differences were clearly significant (Fig. 2). On an average all the varieties under irrigated condition transpired 10–20 g of water per day, while varieties under water stress reduced their water loss to 1–4 g per day. At the end of the experiment P362 variety was found to lose 8.1% water while P1103 and SBD377 showed 12% and 20.2% loss of water compared to their respective controls.

Total leaf area, plant height and chlorophyll content

The total leaf area of a plant represents its photosynthetic area, which is very crucial for the vital activities of the plant. During water stress, plants are affected primarily due to enhanced rate of transpiration, yellowing and finally loss of leaves. The total leaf area of plants of the three varieties was recorded at regular intervals to check the effect of water stress and it was recorded that P362 showed minimum loss of leaves (59%) than P1103 (81%) and SBD377 (91%). While, in control conditions, the number of leaves increased throughout the experiment in all three varieties (Fig. 3A, B). Plant height was also measured at regular intervals. Plants under water stress attained height in the first week, but remained constant in the remaining days of the experiment. This was not the case with well watered controls, as they tend to consistently gain height (Fig. 3C, D).

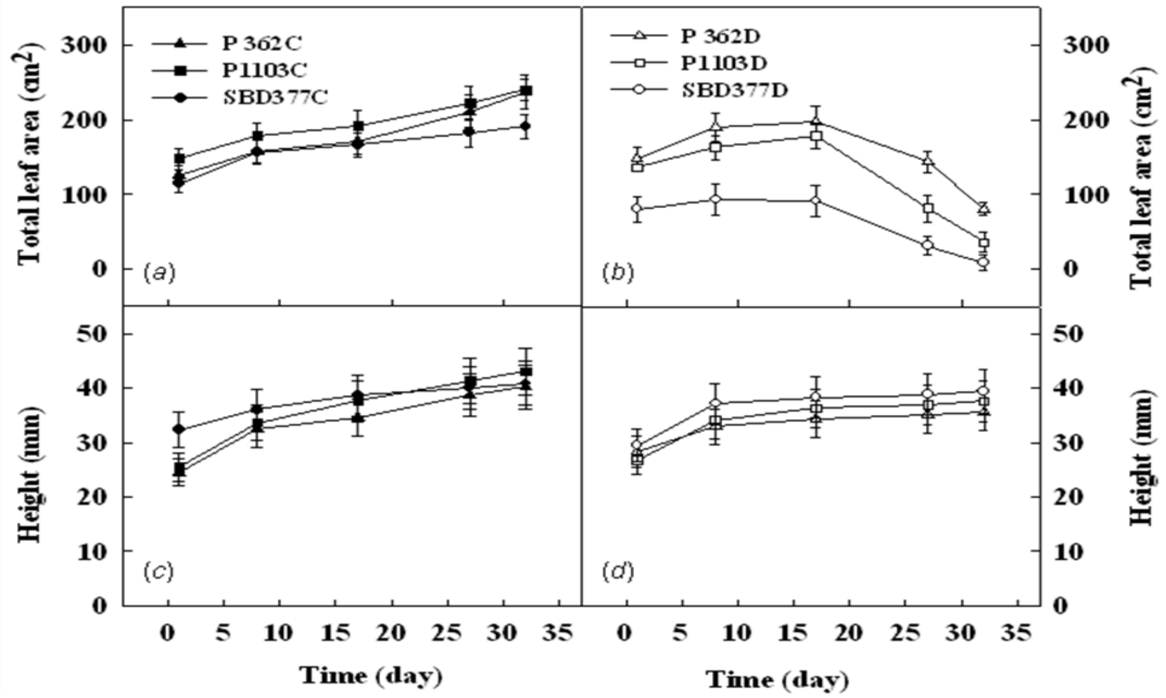

Fig. 3 Total leaf area (cm²) and plant height (mm) measurement under control and drought conditions of three chickpea varieties. (*a*, *c*) P362, P1103 and SBD377 varieties under irrigated conditions and (*b*, *d*) under water stress. Each value represents an average data of ten chickpea plants under the same condition.

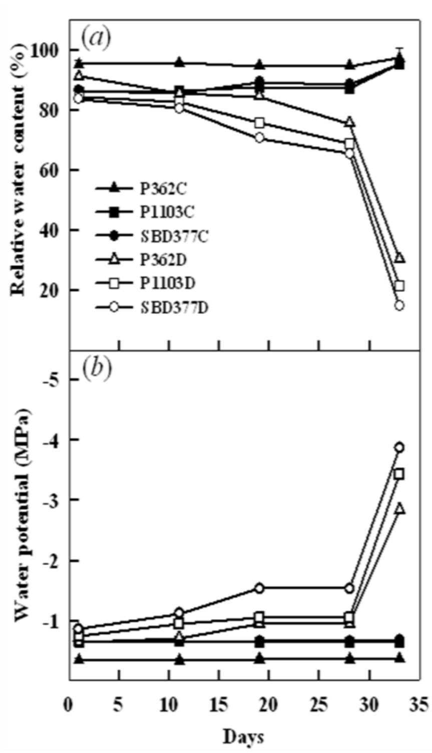

Fig. 4 Changes with time in (*a*) leaf relative water content (RWC) and (*b*) leaf water potential (MPa) in three chickpea varieties under control (-△- -□- -○-) and drought (-▲- -■- -●-) conditions. Each value represents mean of five replicates of ten plants of each variety.

Relative water content (RWC) and water potential (WP)

One of the most common mechanisms by which the plants respond to water limitation is stomatal closure, which reduces water loss and regulates plant water potential (Lawlor 1995). The relative water content of control plants showed a value of 85–95% in all varieties under irrigated conditions. The reduction in RWC value for P362, P1103, and SBD377 was 67, 73 and 81% respectively in one month of drought experiment (Fig. 4A). The WP values among the controls varied from -0.3 to -0.6 MPa in irrigated conditions. Under drought conditions, water potential continuously decreased in all the three varieties, but during the last stage of drought, P362 variety was stable at -2.8 MPa, while it decreased to -3.4 MPa in P1103 and SBD377 showed a WP value of -3.8 MPa (Fig. 4B).

Pressure volume derivatives under irrigated conditions

P362 variety showed maximum osmotic potential at full turgor (-1.01 MPa) due to the high level of effective solute concentration (3.33). This observation was also confirmed by symplastic water content which was 88.40% in P362 compared to 75.60% and 75.20% in P1103 and SBD377 respectively (Fig. 5).

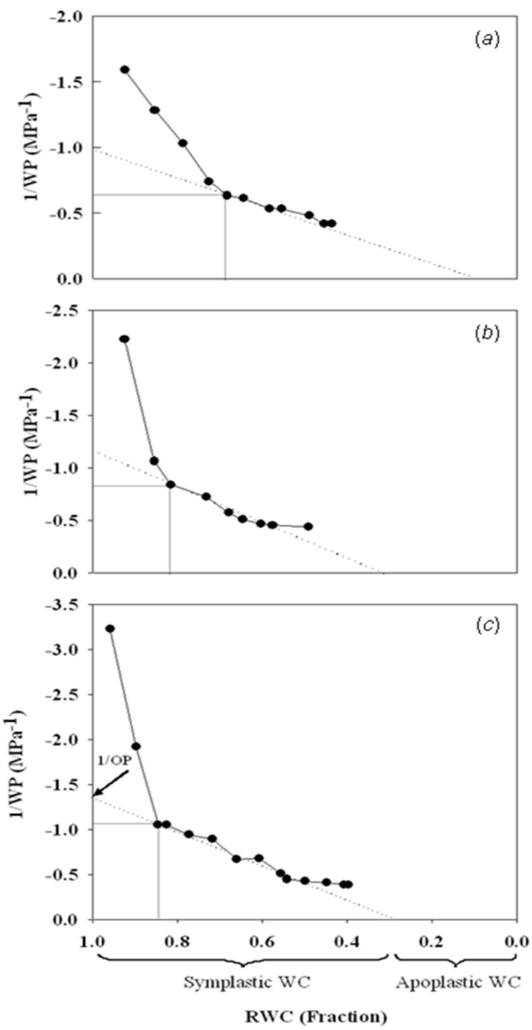

Fig. 5 Typical pressure volume curves of three chickpea varieties under irrigated condition. (*a*) P362, (*b*) P1103 and (*c*) SBD377. OP denotes osmotic potential and WC denotes water content.

A significant difference was observed in elasticity modulus (**ε**) which shows maximum elasticity of the cells in P362 variety. The water potential at turgor loss point in P362 was found to be -1.48 MPa which is significantly higher as compared to P1103 and SBD377 showing -0.97 and -0.95 MPa respectively. The results obtained for different pressure volume derivatives in leaves of three chickpea varieties under irrigated conditions are summarized in Table 1.

Gas exchange and fluorescence estimation

Photosynthesis rate was measured at 1, 15 and 31 days of drought. On imposing drought in P362 variety, photosynthesis rate increased slightly on the first day, which was not observed in the other two varieties. After 15 days of drought, the photosynthesis rate in P362 variety was 11.5 µmol m^{-2} s^{-1} which was 2.5 and 8 folds that of P1103 and SBD377 respectively, while after 1 month, the photosynthesis rate of P362 variety was 2.5 µmol m^{-2} s^{-1} which was 4.2 and 17.5 folds to that of P1103 and SBD377 respectively (Fig. 6A). The transpiration rate in P362 variety under water stress was similar to those under irrigated conditions on the first day of drought experiment, while in other two varieties it increased to 1.5 and 1.3 folds to that of their respective irrigated counterparts. After 15 days a significant reduction in transpiration rate was recorded in all the three varieties under test. After one month, minimal reduction in transpiration rate was observed in P362 variety, while maximum reduction was measured in variety SBD377, compared to their respective controls (Fig. 6B).

The water use efficiency (WUE) is a critical parameter which reveals the state of the plant in bringing carbon dioxide (CO_2) for photosynthesis without losing water through its stomata. In case of P362 variety, the water use efficiency gradually increased up to 1.4 folds in comparison to the plants under control. While WUE of P1103 variety was almost similar to that of control for the first 15 days, but it decreased to 1.3 folds to that of their respective controls. In case of SBD377, value of WUE gradually decreased throughout the experiment and reached half the value of control plants at the end of the experiment (Fig. 6C). The ratio of internal CO_2 concentration and ambient CO_2 concentration (Ci/Ca) values of P362 variety decreased throughout the experiment, but the value of P1103 did not show any significant difference at the end of the experiment. While, in case of SBD377 the Ci/Ca value showed an increasing trend till the last observation as compared to its control (Fig. 6D). The photochemical quantum yield of P362 was initially 1.1 folds to that of P1103 and the difference increased up to 1.7 folds after one month of drought experiment. Whereas, initial photochemical quantum yield of SBD377 variety was 2.1 folds less and up to 5.8 folds lesser than P362 variety after one month of the experiment (Fig. 6E). The electron transport rate was significantly decreased in all the three varieties under water stress and the observed values in P362, P1103 and SBD377 varieties were 1.7, 2.5 and 7.6 fold compared to their respective controls (Fig. 6F).

Maximum photochemical quantum yield is a crucial parameter which represents the overall health of the leaf and reflects maximum efficiency of PSII reaction centre of photosynthesis. There was no significant difference observed in maximum quantum yield of PSII (Fv/Fm) value in the initial stage, during onset of drought among all the three varieties, but towards the completion of the experiment the Fv/Fm value decreased significantly in all the three varieties (Fig. 6G). Non-photochemical quenching (NPQ) indicates a change in the efficiency of excess excitation energy dissipation as heat from the leaf surface. The NPQ value of P362, P1103 and SBD377 variety was found to increase by 1.7, 1.8 and 2 folds in comparison to their respective controls, during the drought experiment (Fig. 6H).

Table 1. Pressure volume derivatives for leaves of three chickpea varieties under irrigated condition.

Parameters	Chickpea varieties		
	P362	**P1103**	**SBD377**
Osmotic potential at full turgor (MPa)	-1.01 ± 0.07	-0.86 ± 0.03	-0.80 ± 0.178*
Symplastic fraction (%)	88.40 ± 2.19	75.6 ± 3.28	75.20 ± 3.03*
Apoplastic fraction (%)	11.60 ± 2.19	24.4 ± 3.28	24.80 ± 3.03*
Elasticity modulus, **ε** (MPa)	2.20 ± 0.33	3.13 ± 0.42	3.28 ± 0.18
Turgor loss point (MPa)	-1.48 ± 0.06	-0.97 ± 0.03	-0.95 ± 0.05*
Water content at turgor loss point (%)	68.40 ± 2.97	82.8 ± 1.10	83.20 ± 1.19*
Effective solute concentration	3.33 ± 1.00	3.09 ± 0.56	2.97 ± 0.34*
Osmotic adjustment (1/p)	0.27 ± 0.04	0.22 ± 0.04	0.21 ± 0.03*

*denotes significant difference between SBD377 and P362 variety. $p \leq 0.05$ according to Student's *t*-test. Osmotic adjustment was assessed as a capacity factor (rate of change in turgor pressure with RWC), by regression of RWC versus turgor pressure. The reciprocal of slope (p) is, therefore, a measure of osmotic adjustment.

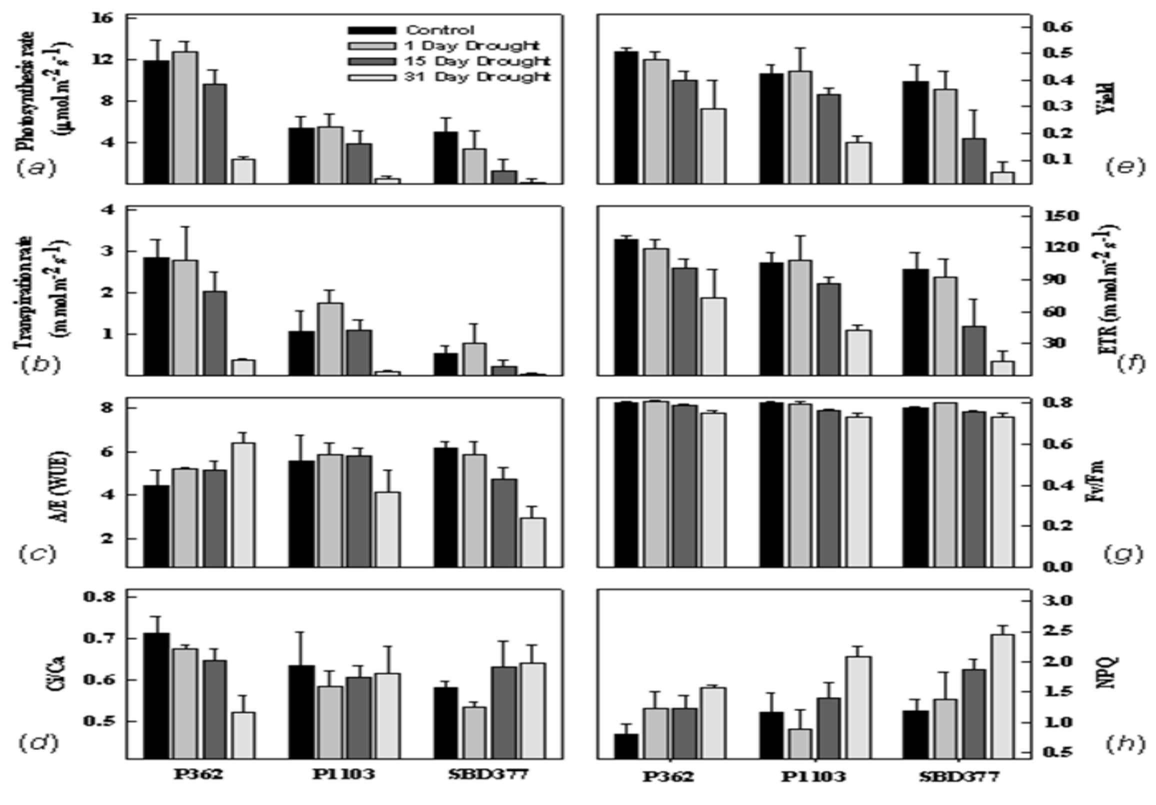

Fig. 6 Leaf gas exchange fluorescence parameters values of three chickpea varieties under control and water stressed conditions. (*a*) photosynthesis rate (A), (*b*) transpiration rate (E), (*c*) water use efficiency (WUE), (*d*) Ci/Ca, (*e*) yield, (*f*) electron transport rate (ETR), (*g*) Fv/Fm and (*h*) NPQ. The readings were taken thrice at 0, 15 and 31 days of drought. Each value is mean of five replicates.

The chlorophyll content was measured on 15th day of drought experiment (Table 2). SBD 377 variety showed 67% reduction in chlorophyll a (*chl* a) content followed by P1103 and P362 with 50% and 30% respectively. A similar trend was observed in chlorophyll b (*chl* b) content with 67, 50 and 30% reduction in SBD377, P1103 and P362 varieties respectively. No significant difference was found in *chl* a/*chl* b ratio in irrigated controls and the plants under test.

Table 2. Drought stress induced changes in leaf chlorophyll content after 15 days of drought (mg g^{-1} fresh weight) in three varieties of chickpea.

Pigment (mg g^{-1} FW)	P362		P1103		SBD377	
	Control	**Drought**	**Control**	**Drought**	**Control**	**Drought**
Chlorophyll a	0.13 ± 0.02	0.09 ± 0.02	0.12 ± 0.04	0.07 ± 0.03	0.15 ± 0.01	0.05 ± 0.01
Chlorophyll b	0.10 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.05 ± 0.02	0.12 ± 0.02	0.04 ± 0.01
Chlorophyll a/b	1.28 ± 0.05	1.24 ± 0.18	1.23 ± 0.15	1.23 ± 0.22	1.26 ± 0.08	1.24 ± 0.32

Data represent the means ± SD of five replicates. $p \leq 0.05$ according to Student's *t*-test.

Soil water parameters

The soil water characteristics were measured by means of WET-sensor (Delta-T Devices, Cambridge, UK). The percent volumetric water content (θ) values (% VWC) showed higher decrease in soil supporting the growth of SBD377, than the other two varieties, during water stress conditions and can be represented as P362 > P1103 > SBD377 (Fig. 7A). Bulk soil electrical conductivity (EC_b) being the total electrical conductivity of the soil, is a function of pore water conductivity, soil particle conductivity, soil moisture content and soil composition. EC_b values of soil in which P362 was grown, showed lesser decrease than the other two varieties, during water stress experiment (Fig. 7B). Permittivity (ε) values of the experimental pot soil was also measured and results showed similar trend, as observed for EC_b values (Fig. 7C).

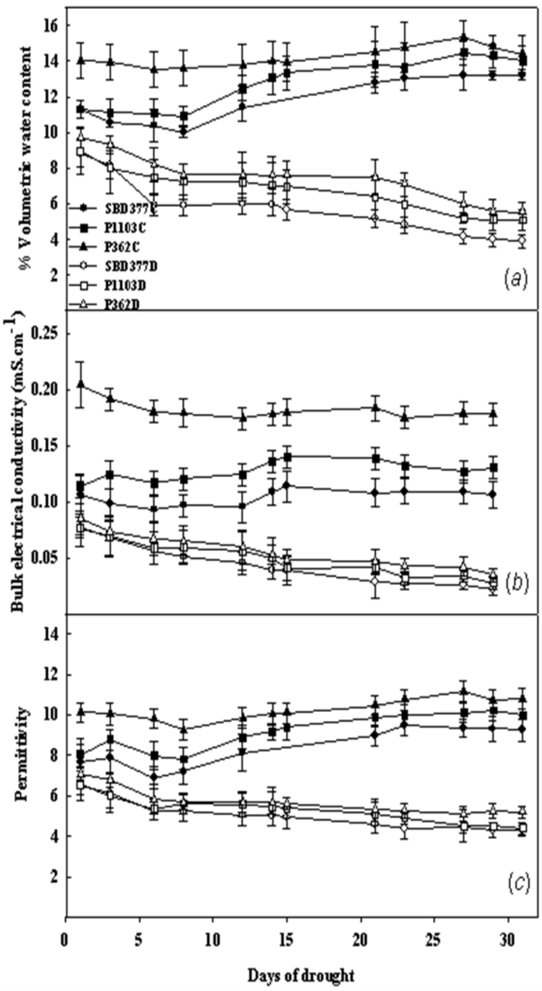

Fig. 7 Effect of drought on soil parameters measured by means of WET sensor (*a*) % volumetric water content (θ), (*b*) bulk electrical conductivity (EC_b), (*c*) permittivity value (mS cm^{-1}). Each reading represents an average value for ten pots under control or drought conditions and repeated thrice. Solid blocks represent control conditions and open blocks represents water stressed conditions.

Discussion

The growth and productivity of crop plants depends largely on their vulnerability to environmental stresses. High salinity, water deficit and temperature stress are the major constrains that limit agricultural production (Araus *et al.,* 2002). Plants respond to these conditions with an array of biochemical and physiological adaptations, which involve the function of many stress-related genes and expression of specific proteins. Hence any attempt to improve stress tolerance requires a better understanding of the physiological, biochemical and molecular events during stress conditions.

The first stress symptom induced by drought in plants, is the rapid inhibition of shoot and root growth. In our case, the average plant height increased in the first 10 days of drought and remained constant thereafter. Similar observations was recorded with average leaf area which increased in the first 15–20 days and finally decreased during the course of the experiment, in all the three varieties under drought (Fig. 3). The stress symptoms are closely followed by partial or complete stomatal closure, with reduction in transpiration and CO_2 uptake for photosynthesis. If not relieved, drought then leads to interrupted reproductive development, premature leaf senescence, wilting and desiccation which culminate in death of the plant (Schulze 1986). P362 variety under drought has shown maximum adaptation to water stress as evident from % RWC status: P362 > P1103 > SBD 377 and WP status: P362 < P1103 < SBD377. In P362 variety, the effective solute concentration and osmotic adjustment was found to be maximum (Table 1) and so it retained its turgidity even after 40% of water loss (60% RWC). Similar results have also been reported in *Brassica* and common bean (Kumar and Singh 1998; Güler *et al.,* 2012).

Photosynthesis rate (A) and transpiration rate (E) were lowered under drought condition in all the three varieties. Highly significant correlation was noted between photosynthesis rate and transpiration rate under drought condition in P362 variety while, in other two varieties correlation between these traits was much less. In P362 variety under drought condition, the Ci/Ca value strongly correlated with photosynthesis rate, which may be due to stomatal limitations on both traits. However, in other two varieties correlation between these traits was poor which shows a poor stomatal regulation. Strong stomatal regulation has helped P362 variety for drought tolerance, as reported earlier in wheat (Monneveux *et al.,* 2006). Fluorescence parameters like photochemical quantum yield, electron transport rate (ETR) and maximum photochemical quantum yield (Fv/Fm) was also found to decrease in all the three varieties but % loss in these parameters was minimal in case of P362 variety (Fig. 6). Similar observations have been reported earlier in cotton (Deeba *et al.,* 2012) and chickpea (Kalefetoglu and Ekmekci *et al.,* 2009). The Fv/Fm value is very sensitive to abiotic stresses like salt, drought, heat and cold stress (Frachebound *et al.,* 1999; Lu and Zhang, 1999; Tezara *et al.,* 2003; Oukarroum *et al.,* 2007). The inhibitory effect of drought on photosynthetic activity has been widely described and is mainly associated with stomatal function and metabolic limitations (Giardi *et al.,* 1996; Lawlor and Tezara 2009). Under conditions of drought stress, leaves experience a transient decrease of Φ PSII, called down-regulation of photochemistry, or they undergo photoinhibition, with decrease in leaf Fv/Fm associated with damage in D1 protein of PSII complex (Osmond 1994). Condition of decreased Φ PSII activity is associated with increase in non-photochemical quenching (NPQ) (Deeba *et al.,* 2012), a protective process which dissipates energy as heat (Maxwell and Johnson 2000; Baker *et al.,* 2007). A minimum reduction in A and a minimal increase in NPQ in P362 variety under drought conditions, suggests that antioxidant defense system and secondary metabolic pathways are enhanced in response to water stress for drought tolerance. Similar results have been reported earlier in other crop plants (Frachebound *et al.,* 1999; Lu and Zhang, 1999; Gill and Tuteja 2010).

Massacci *et al.,* 2008 have observed in *Gossypium hirsutum* that the photosynthesis rate did not vary, while the ETR showed an increase with the onset of drought stress and they attributed it to increase in photorespiration. However, in the present study, the chickpea variety P362 showed a decrease in gas-exchange as well as in the electron transport rate and Φ PSII. Under water stress, when the use of absorbed light in either photosynthesis or photorespiration and the thermal dissipation are not enough to cope with excess energy, the production of highly reactive molecules is exacerbated. These molecules generated within the chloroplast, can cause oxidative damage to the photosynthetic apparatus (Dietz and Pfannschmidt 2011). The decrease in leaf relative water content and the decrease in leaf water potential minimize evapo-transpiration (Fig. 4). Though, the photosynthesis rate was decreased during drought experiment in all the three varieties however, the rate of decrease in photosynthesis was comparatively low in variety P362. Thus, WUE gradually increased in P362 variety, contributing to its enhanced tolerance to drought.

WET-sensor was used to measure soil volumetric water content (θ) and electrical conductivity (EC) in soil. The major advantage of this sensor is that it can measure the two most valuable parameters of irrigation and soil fertility simultaneously. Bulk electrical conductivity (EC_b) reflects the total EC of the entire soil matrix containing soil particles, water, nutrients and air. Though, all the three varieties faced a decrease in soil EC_b value throughout the experiment however, a comparatively lesser decrease was observed in P362 variety (Fig. 7b). The apparent electrical conductivity (ECa) measurements can be used to evaluate the spatial variation in overall quality physico-chemical properties of soil that affect plant yield (Corwin *et al.,* 2003). The application of ECa measurements to precision agriculture plays a crucial role as a viable and sustainable means for meeting the world's future demands for food. A similar decreasing trend in soil permittivity was observed as in ECb values, under drought, but P362 variety faced a lesser reduction than the other two varieties. Permittivity has become a well established method for the determination of the water content of soils, because the real permittivity of water is ~80 at 20 MHz, 25 °C, whereas the permittivity of most soil particles is typically in the range 3 to 8 (Kupfer 2005).

Conclusion

The results of this study indicate that P362 variety has the inherent ability to sense drought at a much earlier stage and responds in a much more efficient manner than P1103 and SBD377 varieties. P362 is a wilt resistant chickpea variety and the water stress tolerant trait is an added asset for introgression of these important traits in other high yielding but drought sensitive varieties of chickpea.

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References

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiology **24**, 1–15.

Araus JL, Slafer GA, Reynolds MP, Royo C. 2002. Plant breeding and drought in C-3 cereals: what should we breed for? Annals of Botany **89**, 925–940.

Baker NR, Harbinson J, Kramer DM. 2007. Determining the limitations and regulation of photosynthetic energy transduction in leaves. Plant Cell and Environment **30**, 1107–1125.

Beckett RP. 1997. Pressure-volume analysis of a range of poikilohydric plants implies the existence of negative turgor in vegetative cells. Annals of Botany **79**, 145–152.

Behboudian MH, Ma Q, Turner NC, Palta JA. 2001. Reactions of chickpea to water stress: yield and seed composition. Journal of the Science of Food and Agriculture **81**, 1288–1291.

Bhatnagar-Mathur P, Vadez V, Jyostna Devi M, Lavanya M, Vani G,Sharma KK. 2009. Genetic engineering of chickpea (*Cicer arietinum* L.) with the *P5CSF129A* gene for osmoregulation with implications on drought tolerance. Molecular Breeding **23**, 591–606.

Corwin DL, Lesch SM, Shouse PJ, Soppe R, Ayars JE. 2003. Identifying soil properties that influence cotton yield using soil sampling directed by apparent soil electrical conductivity. Agronomy Journal **95** (2), 352–364.

Deeba F, Pandey A K, Ranjan S, Mishra A, Singh R, Sharma Y K, Shirke PA, Pandey V. 2012. Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress. Plant Physiology and Biochemistry **53**, 6–18.

Dietz KJ, Pfannschmidt T. 2011. Novel regulators in photosynthetic redox control of plant metabolism and gene expression. Plant Physiology **155**, 1477–1485.

Dita MA, Rispail Prats NE, Rubiales D, Singh KB. 2006. Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica **147**, 1–24.

F.A.O. Statistical Database FAOSTAT Agriculture data. 2012. URL http://faostat.fao.org/faostat.

Frachebound Y, Haldimann P, Leipner J, Stamp P. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). Journal of Experimental Botany **50**, 1533–1540.

Giardi MT, Cona A, Geiken B, Kucera T, Masojidek J and Mattoo AK. 1996. Long-term drought stress induces structural and functional reorganization of photosystem II. Planta **199**, 118–125.

Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry **48**, 909–930.

Güler NS, Sağlam A, Demiralay M and Kadioğlu A. 2012. Apoplastic and symplastic solute concentrations contribute to osmotic adjustment in bean genotypes during drought stress. Turkish Journal of Biology **36**, 151–160.

Jain. 2013. A draft genome sequence of the pulse crop chickpea (*Cicer arietinum* L.). Plant Journal **74**, 715–729.

Kalefetoglu T and Ekmekci Y. 2009. Alterations in photochemical and physiological activities of chickpea (*Cicer arietinum* L.) cultivars under drought stress. Journal of Agronomy and Crop Science **195**, 335–346.

Kumar A, Singh DP. 1998. Use of physiological indices as a screening technique for drought tolerance in oilseed *Brassica* species. Annals of Botany **81**, 413–420.

Kupfer K. 2005. 'Electromagnetic aquametry: Electromagnetic wave interaction with water and moist substances'. (Springer: Berlin, Heidelberg, New York).

Lawlor DW, Tezara W. 2009. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Annals of Botany **103**, 561–579.

Lawlor DW. 1995. Effects of water deficit on photosynthesis. In 'Environment and plant metabolism'. (Eds N Smirnoff) pp. 129–160. (Bios Scientific Publishers Ltd.: Oxford)

Leport L, Turner NC, Davies SL, Siddique KHM. 2006. Variation in pod production and abortion among chickpea cultivars under terminal drought. European Journal of Agronomy **24**, 236–246.

Lu C, Zhang J. 1999. Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. Journal of Experimental Botany **50**, 1199–1206.

Massacci A, Nabiev SM, Pietrosanti L, Nematov SK, Chernikova TN, Thor K, Leipner J. 2008. Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas exchange analysis and chlorophyll fluorescence imaging. Plant Physiology and Biochemistry **46**, 189–195.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany **51**, 659–668.

Monneveux P, Rekika D, Acevedo E, Merah O. 2006. Effect of drought on leaf gas exchange, a carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. Plant Science **170**, 867–872.

Morgan JM, Rodriguez-Maribona B, Knights EJ. 1991 Adaptation to water-deficits in chickpea breeding lines by osmoregulation: relationship to grain-yield in the field. Field Crops Research **27**, 61–70.

Osmond CD. 1994. What is photoinhibition? Some insights from the comparisons of sun and shade plants. In: 'Photoinhibition of photosynthesis-from molecular mechanisms to the field'. (Eds NR Baker, JR Bowyer) pp. 1–24. (BIOS: Oxford)

Oukarroum A, Madidi SE, Schansker G, Strasser RJ (2007) Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and re-watering. Environmental and Experimental Botany **60**, 438–446.

Phogat BS, Singh DP, Singh P. 1984. Response of cowpea (*Vigna sinensis* (L.) Walp.) and Mungbean (*Vigna radiata* (L.) Wilczek to irrigation. Irrigation science **5**, 47–60.

Schulze ED. 1986. Carbon dioxide and water vapor exchange in response to drought in the atmosphere and soil. Annual Review of Plant Physiology **37**, 247–274.

Sheldrake R, Saxena NP. 1979. Growth and development of chickpeas under progressive moisture stress. In 'Stress Physiology in Crop Plants'. (Eds H Mussell, RC Staples) pp. 465–483. (Wiley: New York)

Singh AK, Sopory SK, Wu R, Singla-Pareek SL. 2012. Transgenic approaches for producing abiotic stress tolerant plants. In 'Abiotic stress adaptation in plants: Physiological molecular genomic foundation'. (Eds A Pareek, SK Sopory, HJ Bohert) pp. 418–438. (Springer: The Netherlands)

Singh KB. 1997. Chickpea (*Cicer arietinum* L.). Field Crops Research **53**, 161–170.

Sojka RE and Parsons JE. 1983 Soybean water status and canopy microclimate relationships at four row spacings. Agronomy Journal **75**, 961–968.

Stadelmann EJ. 1984. The derivation of the cell wall elasticity function from cell turgor potential. Journal of Experimental Botany **35**, 859–868.

Subbarao GB, Johansen C, Slinkard AE, Rao RCN, Saxena NP, Chauhan YS. 1995. Strategies for improving drought resistance in grain legumes. Critical Reviews in Plant Sciences **14**, 169–523.

Tezara W, Martinez D, Rengifo E, Herrera A. 2003. Photosynthetic responses of the tropical spiny shrub *Lycium nodosum* (*Solanaceae*) to drought, soil salinity and saline spray. Annals of Botany **92**, 757–765.

Thomas H. 1997. Drought resistance in plants, in: Basra AS, Basra RK (Eds.), Mechanisms of environmental stress resistance in plants. Harwood Academic Publishers, The Netherlands, pp. 1–42.

Turner NC, Begg JE. 1981. Plant-water relations and adaptation to stress. Plant and Soil **58**, 97–107.

Varshney RK. 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nature Biotechnology **31**, 240–248.

Zhang J, Klueva N, Nguygen HT. 1996. Plant adaptation and crop improvement for arid and semi-arid environments. Proceedings of the fifth international conference on desert development, 12–17.

Zhu JK, Hasegawa PM, Bressan RA. 1997. Molecular aspects of osmotic stress in plants. Critical Reviews in Plant Sciences **16**, 253–277.

Abbreviations

RWC	Relative water content
WP	Water potential
A	Net rate of CO_2 uptake per unit of projected leaf area / Photosynthesis rate (µmol m^{-2} s^{-1})
E	Transpiration rate (mmol m^{-2} s^{-1})
WUE	Water use efficiency (A/E)
Ci	Internal CO_2 concentration (µmol mol^{-1})
ETR	Electron transport rate ($mmol^{-2}$ s^{-1})
NPQ	Non-photochemical quenching
Fv/Fm	Effective quantum yield of photosystem II (PSII)
qP	Photochemical quenching